Natural Innate and Adaptive Immunity to Cancer

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Annu. Rev. Immunol. 2011. 29:235–71

First published online as a Review in Advance on January 5, 2011

The *Annual Review of Immunology* is online at immunol.annualreviews.org

This article's doi: 10.1146/annurev-immunol-031210-101324

0732-0582/11/0423-0235$20.00

Keywords

immunosurveillance, immunoediting, equilibrium, immunotherapy, inflammation, immunosuppression

Abstract

The immune system can identify and destroy nascent tumor cells in a process termed cancer immunosurveillance, which functions as an important defense against cancer. Recently, data obtained from numerous investigations in mouse models of cancer and in humans with cancer offer compelling evidence that particular innate and adaptive immune cell types, effector molecules, and pathways can sometimes collectively function as extrinsic tumor-suppressor mechanisms. However, the immune system can also promote tumor progression. Together, the dual host-protective and tumor-promoting actions of immunity are referred to as cancer immunoediting. In this review, we discuss the current experimental and human clinical data supporting a cancer immunoediting process that provide the fundamental basis for further study of immunity to cancer and for the rational design of immunotherapies against cancer.

INTRODUCTION

Extrinsic tumor suppressor: molecular mechanisms of nontransformed cells used to sense the presence of cancerous cells and restrict their growth

Intrinsic tumor suppressor: molecular mechanism within healthy cells that triggers senescence, repair, or apoptosis in an attempt to prevent transformation

TRAIL: TNF-related apoptosis-inducing ligand

Inflammation: a complex physiological process involving leukocyte infiltration that maintains tissue homeostasis in response to tissue stressors such as infection or damage

The fundamental mechanisms of cellular division and DNA replication carry the inherent danger that the replication machinery will inevitably make mistakes, which could compromise the integrity of the genome and potentially result in cancer formation. Extensive research over the past half-century has revealed cancer to be a genetic disease that arises by an evolutionary process where somatic cells acquire multiple mutations that overwhelm the barriers that normally restrain their uncontrolled expansion. The devastation wreaked by cancer cells can be lethal, but fortunately, numerous intrinsic and extrinsic tumor-suppressor mechanisms exist to prevent their development.

A variety of intrinsic tumor-suppressor mechanisms attempt to repair genetic mutations and will trigger senescence or apoptosis should repairs fail and cellular proliferation become aberrant. Cellular senescence, a state characterized by permanent cell-cycle arrest with specific changes in morphology and gene expression that distinguish it from quiescence (reversible cell-cycle arrest), is induced by numerous cellular proteins (e.g., p53) that sense genomic disturbances caused by mutagenic insults (1). In addition, cellular senescence is triggered by activated oncogenes, and it is now becoming more evident that escape from oncogene-induced senescence is a prerequisite for cellular transformation such that cancer cells must acquire cooperating lesions that uncouple mitogenic Ras signaling from senescence to proliferate indefinitely (2). Other intrinsic tumor-suppressor mechanisms, including p53, sense the activity of oncogenes and initiate the programmed cell death machinery. Furthermore, in response to cellular stress, injury, or lack of survival signals, alterations in mitochondria integrity result in the release of proapoptotic effectors that trigger cell death by terminal activation of executioner caspases (3). In contrast, a second cell death pathway is activated through ligation of cell-surface death receptors such as tumor necrosis factor receptor (TNFR), TNF-related apoptosis-inducing ligand (TRAIL) receptor 2 (TRAIL-R2, DR5), and Fas/CD95 (4), with the corresponding ligands of the TNF superfamily inducing the formation of a signaling complex that activates the apical caspase 8 to initiate apoptosis. Furthermore, increasing attention is being placed on alternative cell death pathways such as necrosis, autophagy, and mitotic catastrophe that may halt the transformation process (3). In general terms, both senescence and apoptosis prevent the acquired capability of cells to proliferate without environmental cues and act as a potent barrier to the further development of any preneoplastic cell. These cell-intrinsic prerequisite steps for the transformation of normal cells into cancer cells were graphically illustrated by Hanahan & Weinberg (5), as were sustained angiogenesis, limitless replicative potential, and tissue invasion and metastasis, in the landmark review "The Hallmarks of Cancer."

Since this famous review, at least three general extrinsic tumor-suppressor mechanisms have been identified by which cells and their adjacent tissues sense the presence of cancerous cells. All these, to some extent, can be included under the umbrella of mechanisms that prevent cancer cells from invading and spreading to other tissues in the host. The first rests on the mandatory dependency of cells for specific trophic signals in the microenvironment that quell their innate suicidal tendencies such as the epithelial cell–extracellular matrix association that when disrupted results in cell death (6). A second appears to involve key links between cell polarity genes that control cellular junctions and proliferation, preventing cell cycle progression in the face of dysregulated junctional complexes (7). A third extrinsic tumor-suppressor mechanism involves the limitation of transformation or tumor cell growth by effector leukocytes of the immune system.

The immune system has three primary roles in the prevention of tumors. First, it can protect the host from virus-induced tumors by eliminating or suppressing viral infections. Second, the timely elimination of pathogens and prompt resolution of inflammation can prevent the establishment of an inflammatory environment

conducive to tumorigenesis. Finally, the immune system can specifically identify and eliminate tumor cells in certain tissues on the basis of their expression of tumor-specific antigens (TSAs). This third process, referred to as cancer immunosurveillance, occurs when the immune system identifies transformed cells that have escaped cell-intrinsic tumor-suppressor mechanisms and eliminates them before they can establish malignancy. These effector immune cells employ extremely diverse mechanisms to control tumor targets including the induction of tumor cell death by mitochondrial and cell death receptor pathways, and thus evasion of immunosurveillance is often referred to as the seventh hallmark of cancer (8, 9). In combination, these diverse intrinsic and extrinsic tumor-suppressor mechanisms, which are inexorably linked, are remarkably effective and specific because cancer is relatively rare in long-lived mammals. In this review, we focus on data supporting the conclusion that the immune system acts as an extrinsic tumor suppressor, but paradoxically can also promote cancer initiation, promotion, and progression.

A MODERN HISTORY OF CANCER IMMUNOSURVEILLANCE AND CANCER IMMUNOEDITING

The idea that the immune system, which so effectively protects the host from microbial pathogens, might also recognize and destroy tumor cells was conceived 50–100 years ago (10–12). For more than a century, the concept of cancer immunosurveillance has been wrought with controversy (reviewed in detail in Reference 8), and by the early 1990s, little attention was paid to the idea that natural immunity could eliminate tumors de novo. However, interest in this aspect of tumor immunology was rekindled in the mid-1990s by the observations that transplanted tumors grew more robustly in mice treated with neutralizing monoclonal antibodies specific for interferon-γ (IFN-γ) (13) and that immunodeficient mice that lacked either IFN-γ responsiveness (IFNGR1, a component of the IFN-γ receptor) or an intact T cell compartment were more susceptible to methylcholanthrene (MCA)-induced sarcoma formation (14–16).

In the past decade, work from many laboratories including our own has validated the concept of cancer immunosurveillance, demonstrating unequivocally that the immune system can indeed protect mice from outgrowth of many different types of primary and transplantable tumors (13, 14, 17–21). An important study in 2001 provided evidence that the immune system controlled not only tumor quantity but also tumor quality (i.e., immunogenicity) (17). Immunodeficient mice lacking either IFN-γ responsiveness or recombination activating gene-2 (RAG-2) [the latter fail to generate T, B, and natural killer T (NKT) lymphocytes] develop more spontaneous neoplasia upon aging and are more susceptible to MCA carcinogen-induced sarcomas compared with wild-type mice. In addition, a significant portion (40%) of MCA sarcomas derived from immunodeficient *Rag2*$^{-/-}$ mice was spontaneously rejected when transplanted into naive syngeneic wild-type mice, whereas all MCA sarcomas derived from immunocompetent wild-type mice grew progressively when transplanted into naive syngeneic wild-type hosts (17). Thus, tumors formed in the absence of an intact immune system are, as a group, more immunogenic than tumors that arise in immunocompetent hosts. These results show that the immune system not only protects the host against tumor formation, but also edits tumor immunogenicity. These new data prompted a refinement of the cancer immunosurveillance concept and led to the formulation of the cancer immunoediting hypothesis, which stresses the dual host-protective and tumor-sculpting actions of immunity on developing tumors.

We now view cancer immunoediting as a dynamic process composed of three distinct phases: elimination, equilibrium, and escape (8, 22–26) (**Figure 1**). Elimination is a modernized view of cancer immunosurveillance in which molecules and cells of both innate and adaptive immunity work together to detect the

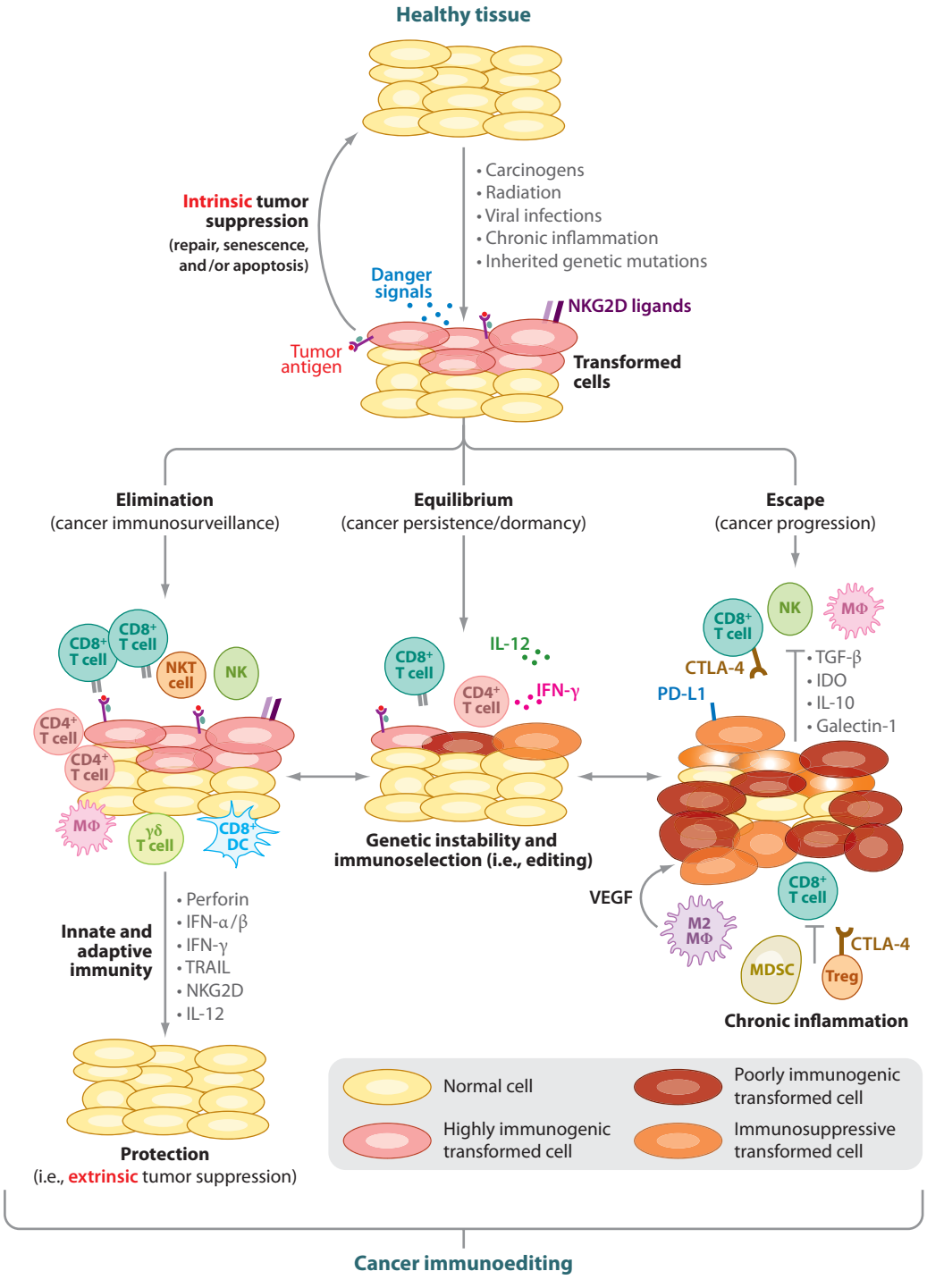
IFN: interferon

Immunodeficient: lacking one or more functional components of the immune system

MCA: methylcholanthrene

RAG: recombination activating gene

Immunoediting: a continual process during tumorigenesis where the immune system both protects against tumor development and promotes their outgrowth



presence of a developing tumor and destroy it long before it becomes clinically apparent. In instances in which tumor cell destruction goes to completion, the elimination phase represents an endpoint of cancer immunoediting. However, tumor cell variants may sometimes not be completely eliminated but rather enter into an equilibrium phase where the immune system controls net tumor cell outgrowth. In this equilibrium phase, tumor cells can become functionally dormant and remain clinically unapparent for the life of the host. Thus, equilibrium also represents a potential second stable endpoint of cancer immunoediting. Finally, either as a result of changes occurring (*a*) in the tumor cell population due to an active immunoediting process or (*b*) in the host immune system, resulting from increases in cancer-induced immunosuppression or immune system breakdown due to the natural aging process, the functional dormancy of the tumor cell population may be broken, leading to progression of these cells into the escape phase, where they begin to grow in an immunologically unrestricted manner and emerge as clinically apparent disease. The concept of cancer immunoediting is thus a comprehensive interpretation of previous and current clinical and experimental data, which integrates the immune system's capacity to both protect the host from cancer and promote cancer outgrowth through a multitude of mechanisms. The observations that have led to the concept of cancer immunoediting are reviewed here, with a particular focus on experimental data from various mouse models of cancer and clinical data from human cancer patients.

Elimination phase: modern view of cancer immunosurveillance where the immune system destroys transformed cells

Equilibrium phase: immune-mediated tumor dormancy

Immunosuppression: the function of one or more components of the immune system is diminished

Escape phase: transformed cells acquire adaptations allowing them to grow unhindered by the immune system

THE ELIMINATION PHASE: CANCER IMMUNOSURVEILLANCE

Immune-Mediated Cancer Elimination in Mice

In the first phase of the cancer immunoediting process, the elimination phase, immune cells locate, recognize, and destroy nascent transformed cells and prevent the development of malignancy. This process has never been visualized in vivo, but rather has been inferred from the earlier onset or greater penetrance of neoplasia in mice defective for certain immune cell subsets, recognition molecules, effector pathways, or cytokines. Predominantly through the use of gene-targeted mice or by employing neutralizing monoclonal antibodies (mAbs) in wild-type mice, this approach has demonstrated that numerous immune effector cells and pathways are important for the suppression of tumor development (**Tables 1–3**). For the purposes of this review, we do not discuss a large literature where such mice have been challenged by transplanting a bolus of tumor cells derived from wild-type mice because these tumor cells originated by escaping host immunity and therefore have already undergone cancer immunoediting. There are three basic mouse models of cancer

Figure 1

The three phases of cancer immunoediting. Cancer immunoediting is the result of three processes that function either independently or in sequence to control and shape cancer. Once normal cells are transformed into tumor cells by the combination of acquired oncogenes and failed intrinsic tumor-suppressor mechanisms, the immune system may function as an extrinsic tumor suppressor by eliminating tumor cells or preventing their outgrowth. In the first phase, elimination, previously known as cancer immunosurveillance, innate and adaptive immune cells and molecules recognize transformed cells and destroy them, resulting in a return to normal physiological tissue. However, if antitumor immunity is unable to completely eliminate transformed cells, surviving tumor variants may enter into the equilibrium phase, where cells and molecules of adaptive immunity prevent tumor outgrowth. These variants may eventually acquire further mutations that result in the evasion of tumor cell recognition, killing, or control by immune cells and progress to clinically detectable malignancies in the escape phase. (Abbreviations: CTLA-4, cytotoxic T lymphocyte associated protein-4; IDO, indoleamine 2,3-deoxygenase; IFN, interferon; IL, interleukin; MΦ, macrophage; MDSC, myeloid-derived suppressor cells; NK, natural killer; NKG2D, NK group 2, member D; PD-L1, programmed cell death 1 ligand 1; TGF-β, transforming growth factor-β; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.) Figure modified from *Nature Immunology* (8).

Table 1 Susceptibility of immunodeficient mice to carcinogen-induced tumors[a]

Mouse strain	Immune status	Tumor susceptibility relative to wild-type mice	References
Rag1$^{-/-}$ *or Rag2*$^{-/-}$	Lacks T cells, B cells, and NKT cells	↑ MCA-induced sarcomas	17, 27
Rag2$^{-/-}$*Stat1*$^{-/-}$	Lacks T cells, B cells, and NKT cells; insensitive to IFN-α, IFN-β, and IFN-γ	↑ MCA-induced sarcomas	17
SCID	Lacks T cells, B cells, and NKT cells	↑ MCA-induced sarcomas	16, 27
Tcrb$^{-/-}$	Lacks αβ T cells	↑ MCA-induced sarcomas	21
Tcrd$^{-/-}$	Lacks γδ T cells	↑ MCA-induced sarcomas	21
		↑ DMBA/TPA-induced skin tumors	
Tcrb$^{-/-}$*Tcrd*$^{-/-}$	Lacks αβ T cells and γδ T cells	↑ DMBA/TPA-induced skin tumors	28
Nude (athymic)	Lacks most T cells	↑ MCA-induced sarcomas	15
Cd1d$^{-/-}$	Lacks CD1d-restricted T cells	↑ MCA-induced sarcomas	29
Ja18$^{-/-}$	Lacks semi-invariant NKT cell subset	↑ MCA-induced sarcomas	19, 27, 203
Ifngr1$^{-/-}$	Insensitive to IFN-γ	↑ MCA-induced sarcomas	14, 17
Ifng$^{-/-}$	Lacks IFN-γ	↑ MCA-induced sarcomas and skin tumors	31, 46, 47
		↑ MNU-induced lymphomas	
Stat1$^{-/-}$	Insensitive to IFN-α, IFN-β, and IFN-γ	↑ MCA-induced sarcomas	14, 17
Ifnar1$^{-/-}$	Insensitive to IFN-α and IFN-β	↑ MCA-induced sarcomas	32
Ifnar2$^{-/-}$	Insensitive to IFN-α and IFN-β	↑ MCA-induced sarcomas	33
Pfp$^{-/-}$	Lacks perforin	↑ MCA-induced sarcomas	31, 204
Pfp$^{-/-}$*Ifng*$^{-/-}$	Lacks perforin and IFN-γ	↑ MCA-induced sarcomas	31
Il12a$^{-/-}$	Lacks IL-12	↑ DMBA/TPA-induced skin tumors	46, 57
		↑ MNU-induced lymphomas	
Il12b$^{-/-}$	Lacks IL-12 and IL-23	↑ MCA-induced sarcomas	19, 57, 58
		↓ DMBA/TPA-induced skin tumors	
Il23a$^{-/-}$	Lacks IL-23	↓ MCA-induced sarcomas	57, 58
		↓ DMBA/TPA-induced skin tumors	
Cd80$^{-/-}$*Cd86*$^{-/-}$	Lacks CD80 and CD86	↑ UV-induced skin tumors	205
NK1.1-specific antibody treatment	Lacks NK cells and NKT cells	↑ MCA-induced sarcomas	18, 27
Asialo-GM1-specific antibody treatment	Lacks NK cells	↑ MCA-induced sarcomas	18, 27
RAE1 transgenic	Defective killing through NKG2D pathway	↑ DMBA/TPA-induced skin tumors	54
Trail$^{-/-}$	Lacks TRAIL	↑ MCA-induced sarcomas	35
TRAIL-specific antibody treatment	Blockade of TRAIL	↑ MCA-induced sarcomas	36
Tnf$^{-/-}$	Lacks TNF-α	↑ MCA-induced sarcomas	34, 49
		↓ DMBA/TPA-induced skin tumors	
Δ*dblGATA*	Lacks eosinophils	↑ MCA-induced sarcomas	30
Ccl11$^{-/-}$*Il5*$^{-/-}$	Lacks eosinophils	↑ MCA-induced sarcomas	30
IL-5 transgenic	Elevated number of eosinophils	↓ MCA-induced sarcomas	30

(*Continued*)

Table 1 (*Continued*)

Mouse strain	Immune status	Tumor susceptibility relative to wild-type mice	References
Myd88$^{-/-}$	Lacks MyD88	↓ MCA-induced sarcomas	34, 194, 195, 206
		↓ DMBA/TPA-induced skin tumors	
		↓ DEN-induced liver carcinomas	
		↓ AOM-induced colon polyps	
		↑ AOM/DSS-induced colon carcinomas	
Il10$^{-/-}$	Lacks IL-10	↓ MCA-induced sarcomas	34
Il1b$^{-/-}$	Lacks IL-1β	↓ MCA-induced sarcomas	192
Dnam1$^{-/-}$	Lacks DNAM-1	↑ MCA-induced sarcomas	38
		↑ DMBA/TPA-induced skin tumors	
Il17a$^{-/-}$	Lacks IL-17A	↓ DMBA/TPA-induced skin tumors	58
CD4, CD25, or FR4 specific antibody treatment	Lacks Regulatory T cells	↓ MCA-induced sarcomas	117, 193
Immunization with self antigen	Increased regulatory T cell-activity	↑ MCA-induced sarcomas	207

[a]Abbreviations: AOM, azoxymethane; *Ccl11*, chemokine (C-C motif) ligand 11; *Cd*, cluster of differentiation; Δ*dblGATA*, deletion of high-affinity GATA binding site in *Gata1* promoter; DEN, diethyl-nitrosamine; *Dnam1*, DNAX accessory molecule-1; DMBA, 7,12-di-methylbenz[a]-anthracene; DSS, dextran sodium sulfate; FR4, folate receptor 4; GM1, a ganglioside; *Ifn*, interferon; *Ifnar1,2*, type I IFN receptor 1,2; *Ifngr1*, IFN-γ receptor 1; *Il*, interleukin; *Ja18*, Jα18 joining gene segment of TCR; MCA, methylcholanthrene; MNU, N-methyl-N-nitrosourea; *Myd88*, myeloid differentiation primary response gene 88; NK, natural killer; NK1.1, NK cell–associated antigen 1.1; NKG2D, NK group 2, member D; NKT, natural killer T; *Pfp*, perforin; RAE, retinoic acid early transcript; *Rag*, recombination activating gene; SCID, severe combined immunodeficient; *Stat1*, signal transducer and activation of transcription 1; *Tcr*, T cell receptor; *Tnf*, tumor necrosis factor-α; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; *Trail*, TNF-related apoptosis-inducing ligand; UV, ultraviolet.

that are relevant to the discussion of cancer immunoediting that illustrate the important role of immunity in eliminating developing tumors: (*a*) carcinogen-induced tumors, (*b*) spontaneous tumors that arise upon aging, and (*c*) tumor development in mice genetically predisposed to cancer.

Carcinogen-Induced Tumors in Immunodeficient Mice

Historically, the concepts of cancer immunosurveillance and immunoediting have predominantly been demonstrated by exposing wild-type and immunodeficient mice to carcinogens and comparing their relative tumor incidences. The advantages of regulating tumor penetrance, tissue involvement, and location in carcinogen-induced tumor models are one reason why these models are widely employed by researchers, and in some cases they represent good mouse models of human cancer (e.g., asbestosis). The two most commonly employed carcinogen-induced tumor models are sarcomas induced using 3′-MCA and skin papillomas induced by a combination of 7,12-di-methylbenz[a]-anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA). To date, numerous mice with defined immunodeficiencies have been tested for their susceptibility to carcinogens (**Table 1**).

Cells of both the innate and adaptive immune system have been shown to be critical for the elimination (i.e., cancer immunosurveillance) of primary MCA-induced sarcomas. Lymphocyte-deficient *Rag1*$^{-/-}$, *Rag2*$^{-/-}$, severe combined immunodeficient (SCID), and nude mice all display an increased susceptibility to tumor induction after MCA exposure (15–17, 27). Interestingly, 40% of

DMBA: 7,12-di-methylbenz[a]-anthracene

TPA: 12-O-tetradecanoyl-phorbol-13-acetate

Table 2 Immunodeficient mice develop spontaneous tumors[a]

Mouse strain	Immune status	Spontaneous tumor development	References
Rag2$^{-/-}$	Lacks T cells, B cells, and NKT cells	Mice develop intestinal and lung neoplasms	17
Rag2$^{-/-}$*Stat1*$^{-/-}$	Lacks T cells, B cells, and NKT cells; insensitive to IFN-α, IFN-β, and IFN-γ	Mice develop intestinal adenomas like *Rag2*$^{-/-}$ mice but also develop mammary and colon adenocarcinomas	17
Ifng$^{-/-}$	Lacks IFN-γ	Mice develop disseminated lymphomas on C57BL/6 background and lung adenocarcinomas on BALB/c background	20
Pfp$^{-/-}$	Lacks perforin	Mice develop B cell lymphomas	18
Pfp$^{-/-}$*Ifng*$^{-/-}$	Lacks perforin and IFN-γ	Mice develop B cell lymphomas similar to *Pfp*$^{-/-}$ mice, but with earlier onset and greater frequency	20
Pfp$^{-/-}$*B2m*$^{-/-}$	Lacks perforin, MHC I molecules, and CD8$^+$ T cells	Mice develop B cell lymphomas similar to *Pfp*$^{-/-}$ mice, but with earlier onset and greater frequency	62
Trail$^{-/-}$	Lacks TRAIL	Mice develop lymphomas	63
gld mutant	Mutant FasL	Mice develop plasmacytomas	64
Lmp2$^{-/-}$	Defective antigen processing	Mice develop uterine neoplasms	68
Gmcsf$^{-/-}$*Ifng*$^{-/-}$	Lacks GM-CSF and IFN-γ	Mice develop a range of malignancies including lymphomas and solid tumors	70
Il12rb2$^{-/-}$	Lacks IL-12Rβ2	Mice develop plasmacytomas and lung carcinomas	69

[a]Abbreviations: *B2m*, beta-2 microglobulin; CD, cluster of differentiation; FasL, Fas ligand; *gld*, generalized disease mutant mice; *Gmcsf*, granulocyte-macrophage colony stimulating factor; *Ifn*, interferon; *Ifng*, IFN-γ; *Il12rb2*, interleukin-12 receptor subunit β2; *Lmp2*, low-molecular-mass protein 2; MHC I, major histocompatibility complex class I; NKT, natural killer T; *Pfp*, perforin; *Rag2*, recombination activating gene 2; *Stat1*, signal transducer and activation of transcription 1; *Trail*, TNF-related apoptosis-inducing ligand.

tumors derived from *Rag2*$^{-/-}$ mice are rejected when transplanted into wild-type recipients, but they grow progressively in either *Rag2*$^{-/-}$ hosts or wild-type hosts depleted of CD4$^+$ and CD8$^+$ T cells, whereas tumors derived from wild-type mice grow readily when transplanted into either wild-type or *Rag2*$^{-/-}$ hosts. These observations demonstrate that carcinogen-induced sarcomas derived from immunodeficient mice are more immunogenic than those arising in mice with a functional immune system, and thus formed the basis for the cancer immunoediting concept (17). Subsequent studies found that mice deficient for either αβ or γδ T cells display increased susceptibility to tumor induction, indicating that both lymphocyte populations are important in suppressing MCA-induced tumors (21, 28). The innate-like lymphocytes are also critical players in eliminating transformed cells. For example, mice lacking CD1d-restricted T cells (*Cd1d*$^{-/-}$) are more susceptible to MCA-induced sarcomas (29), suggesting that these cells, which bridge the innate and adaptive arms of the immune system, also have a role in suppressing MCA-induced sarcomas. Furthermore, mice lacking the Jα18 T cell receptor (TCR) component are unable to generate the semi-invariant Vα14-Jα18-containing TCR expressed by NKT cells, resulting in the absence of NKT cells and rendering these mice more susceptible to MCA-sarcoma induction (19). Consistent with a role for the innate immune cells in cancer immunosurveillance, mice chronically depleted of NK cells displayed increased tumor incidence (27). Even eosinophils, whose role is more clearly defined in host-defense against helminths, can protect the host from tumor development. Mice deficient in eosinophils (more specifically eotaxin-, CCL11-, and/or IL-5-deficient or Δ*dblGATA*) were more susceptible to MCA-induced sarcoma formation than wild-type mice in both C57BL/6 and BALB/c backgrounds (30). Remarkably, mice

Table 3 Immunodeficiency increases the frequency of cancer in genetic mouse tumor models[a]

Mouse strain	Immune status	Spontaneous tumor development	References
Ifngr1$^{-/-}$*Trp53*$^{-/-}$	Insensitive to IFN-γ and lacks p53	Mice develop broader spectrum of tumors with an earlier onset than *Trp53*$^{-/-}$ mice	14
Stat1$^{-/-}$*Trp53*$^{-/-}$	Insensitive to IFN-α, IFN-β, and IFN-γ and lacks p53	Mice develop broader spectrum of tumors with an earlier onset than *Trp53*$^{-/-}$ mice	14
Ifng$^{-/-}$ HTLV-Tax	Lacks IFN-γ and transgenic for human HTLV-Tax	Mice develop leukemia and/or lymphoma with an earlier onset than HTLV-Tax transgenic mice	73
Pfp$^{-/-}$*Trp53*$^{+/-}$	Lacks perforin and is heterozygous for p53	Mice develop B cell lymphomas with an earlier onset than *Trp53*$^{+/-}$ mice	18
Pfp$^{-/-}$ Her2/neu	Lacks perforin on a Her2/neu background	Mice develop mammary adenocarcinomas with an earlier onset than Her2/neu mice	208
Pfp$^{-/-}$ *Mlh1*$^{+/-}$	Lacks perforin and heterozygous for Mlh-1	Mice develop B cell lymphomas with an earlier onset than *Mlh1*$^{+/-}$ mice	72
Pfp$^{-/-}$ Eμ-v-Abl	Lacks perforin and transgenic for Eμ-v-Abl	Mice develop plasmacytomas with an earlier onset than Eμ-v-Abl transgenic mice	72
Pfp$^{-/-}$ vav-bcl2	Lacks perforin and transgenic for vav-bcl2	Mice develop follicular lymphomas with an earlier onset than vav-bcl2 transgenic mice	72
Cd1d$^{-/-}$*Trp53*$^{+/-}$	Lacks CD1d-restricted T cells and is heterozygous for p53	Mice develop lymphomas and sarcomas with an earlier onset than *Trp53*$^{+/-}$ mice	29
Ja18$^{-/-}$*Trp53*$^{+/-}$	Lacks semi-invariant NKT cells and is heterozygous for p53	Mice develop lymphomas and sarcomas with an earlier onset than *Trp53*$^{+/-}$ mice	29
Ja18$^{-/-}$ TRAMP	Lacks semi-invariant NKT cells on TRAMP background	Mice develop more aggressive prostate carcinomas with an earlier onset than TRAMP mice	75
Trail$^{-/-}$*Trp53*$^{+/-}$	Lacks TRAIL and is heterozygous for p53	Mice develop B cell lymphomas and sarcomas with an earlier onset than *Trp53*$^{+/-}$ mice	63
Trailr$^{+/-}$ Eμ-myc	Heterozygous for TRAIL receptor and transgenic for Eμ-myc	Mice develop lymphomas and metastases with an earlier onset than transgenic Eμ-myc mice	74
Klrk1$^{-/-}$ TRAMP	Lacks NKG2D on TRAMP background	Mice develop larger prostate carcinomas with an earlier onset than TRAMP mice	40
Klrk1$^{-/-}$ Eμ-myc	Lacks NKG2D and transgenic for Eμ-myc	Mice develop lymphomas with an earlier onset than Eμ-myc transgenic mice	40

[a]Abbreviations: *Cd1d*, cluster of differentiation 1d; Eμ-myc, myelocytomatosis oncogene regulated by immunoglobulin heavy chain enhancer-promoter; Eμ-v-Abl, Abelson murine leukemia viral oncogene regulated by Eμ; vav-bcl2, B cell leukemia/lymphoma 2 regulated by the panhematopoietic promoter, vav; *Ifn*, interferon; *Ifng*, IFN-γ; *Ifngr1*, IFN-γ receptor 1; Her2/neu, human epidermal growth factor receptor 2; HTLV, human T cell leukemia virus; *Ja18*, Jα18 joining gene segment of TCR; *Klrk1*, killer cell lectin-like receptor subfamily K, member 1; *Mlh1*, mutL homolog 1; NKG2D, NK group 2, member D; NKT, natural killer T; *Pfp*, perforin; *Stat1*, signal transducer and activation of transcription 1; *Trail*, TNF-related apoptosis-inducing ligand; *Trailr*, TRAIL receptor; TRAMP, transgenic adenocarcinoma of the mouse prostate; *Trp53*, transformation related protein 53.

transgenic for IL-5 have greater circulating numbers of eosinophils and were more resistant to MCA sarcomas compared with wild-type mice, strongly suggesting an immunosurveillance role for these innate immune cells (30).

Numerous mice deficient for specific immune effector molecules and recognition pathways have also been examined in the context of MCA-induced tumor susceptibility, including mice lacking perforin (31), IFN-γ (31), IFNGR1 (14, 17), IFNAR1 or IFNAR2 (components of the type I IFN receptor) (32–34), TRAIL (35, 36), IL-12 (37), TNF-α (34), and DNAM-1 (DNAX accessory molecule-1) (38). Each of these mouse strains demonstrated enhanced susceptibility to sarcoma induction after

NKG2D: NK group 2, member D

MCA treatment, suggesting that IFNs and cytotoxic lymphocytes suppress tumor initiation in vivo. Although treatment of wild-type mice with blocking antibodies specific for NKG2D (an activating receptor expressed by $CD8^+$ T cells, γδ T cells, and NK cells) was reported to increase the incidence of MCA-induced sarcomas in two different mouse strains (39), C57BL/6 NKG2D-deficient mice had comparable numbers of MCA-induced sarcomas to wild-type mice (40). In addition, although the rate of MCA-induced tumor formation was similar in the presence or absence of the NK cell natural cytotoxicity receptor NKp46, the expression of its unknown ligands was NKp46-dependent, suggesting some level of immunoediting by cells expressing this receptor (41).

IFNs can contribute to antitumor effects in a number of ways. IFN-γ can exert direct effects on tumor cells (14), and a major effect of IFN-γ on these cells is to enhance MHC class I expression, rendering them better targets for tumor-specific $CD8^+$ T cells (17, 42). In addition, IFN-γ signaling in host immune cells (43) and host stroma cells (44) plays an important role in the elimination of tumor cells, indicating that IFN-γ's effects in multiple cellular compartments generates antitumor immunity. Others have proposed that IFN-γ contributes to an inflammatory foreign body reaction that results in the encapsulation of injected MCA, limiting its spread and thereby reducing its carcinogenic effects (45). However, this mechanism does not explain the findings that IFN-γ prevents the formation of lymphomas induced by the soluble carcinogen N-methyl-N-nitrosourea (46), where encapsulation of the carcinogen is not possible. Furthermore, a recent report demonstrated that MCA exposure induced more squamous cell carcinomas (SCCs) in the skin of IFN-γ-deficient mice than of wild-type controls (47), indicating that MCA delivery in a different tissue type than the previous subcutaneous injections also supports a role for IFN-γ in mediating cancer immunosurveillance. In contrast to the antitumor effects of IFN-γ occurring at the levels of both the tumor and the host, the antitumor effects of type I IFNs (IFN-α/β) are mediated only at the level of the host's hematopoietic system (32). These results suggest that the ability of type I IFNs to induce antitumor activity in immune cells might be the critical mode of action for this cytokine family and that IFN-γ and IFN-α/β have distinct, potentially nonoverlapping mechanisms of action in antitumor immunity.

As discussed later in this review, recent studies reveal that initiation of MCA-induced sarcomas requires an inflammatory event. Along similar lines, skin carcinomas induced by the topical application of DMBA (tumor initiator) followed by repetitive doses of TPA (tumor promoter) are known to require inflammatory components for tumor initiation and promotion. For example, the induction of DMBA/TPA skin carcinomas is myeloid differentiation primary response gene 88 (MyD88)- (34), mitogen-activated protein kinase-activated protein kinase-2 (MK2)- (48), TNF-α- (49), receptor for advanced glycation end-products (RAGE)- (50), and indoleamine 2,3-deoxygenase (IDO)-dependent (51). Lesions progress from benign papillomas to metastatic SCC, and both the number of lesions and extent of tumor progression is dependent on the mouse strain. Despite an inflammatory component, DMBA/TPA-induced tumors are also detected and destroyed by effector cells and molecules of innate and adaptive immunity. For example, γδ T cells and $CD8^+$ T cells confer protection from DMBA/TPA-induced papillomas (21, 52). In contrast, $CD4^+$ T cells promote tumor progression, implying opposite roles for αβ T cell subsets in the protection or promotion of DMBA/TPA skin carcinogenesis (52). One mechanism by which γδ T cells and activated $CD8^+$ T cells might regulate tumor development is through recognition by NKG2D of the stress ligand retinoic acid early transcript 1 (RAE1) that is induced in the skin after DMBA/TPA treatment and is upregulated in transformed cells by the DNA damage pathway (21, 53). NKG2D-expressing dendritic epidermal γδ T cells can kill RAE1-expressing targets in vitro (21), but in transgenic mice expressing RAE1 in the skin, NKG2D expression

is downmodulated on lymphocytes, and consequently these mice are more susceptible to papilloma induction than wild-type mice (54). A follow-up study using inducible RAE1 transgenic mice has provided further insight into the previous observation, where acute upregulation of NKG2D ligands triggered a swift reorganization of the local skin immune compartment, resulting in local $V\gamma5V\delta1^+$ T cells limiting carcinogenesis, but unexpectedly Langerhans cells promoted DMBA/TPA carcinogenesis (55). Another innate recognition receptor, DNAM-1, also protects tumor formation as *Dnam1*$^{-/-}$ mice develop more papillomas than their wild-type counterparts (38).

In addition to cellular subsets and recognition receptors, effector molecules and cytokines have a critical function in controlling DMBA/TPA-induced skin tumors. For example, although DMBA/TPA-treated TRAIL-R-deficient mice did not show an increase in the number of benign papillomas or the rate of progression to SCC when compared with wild-type mice, metastasis to lymph nodes was significantly enhanced, indicating a role for TRAIL-R specifically in the suppression of metastasis (56). One cytokine, IL-12, has been shown to protect mice against DMBA/TPA-induced tumors, whereas mice that lack functional IL-12 (*Il12a*$^{-/-}$) develop increased numbers of papillomas compared with wild-type mice (57, 58). Interestingly, mice that lack functional IL-23 (*Il23a*$^{-/-}$) are resistant to tumor development (57, 58); however, the mechanism by which IL-23 suppresses innate immunity and promotes tumor growth requires further clarification because it was unexpectedly IL-17A-independent (58). Nevertheless, IL-17A-deficient mice also develop fewer skin papillomas than wild-type mice after DMBA/TPA exposure, suggesting a tumor-promoting role for this cytokine (58). One peculiarity of the DMBA/TPA model is that a loss of IFN-γ or IFNGR1 unexpectedly results in reduced tumorigenesis, hence playing an opposite role than in the MCA tumor model (59). These observations demonstrate the pleiotropic effects that a single immune cell or molecule can have during carcinogenesis and stress the importance of a multimodal analysis. The interplay between antitumor immunity and cancer-promoting inflammation suggested by the above studies is discussed at greater length below.

In addition to the demonstration of cancer immunosurveillance by immune effector cells and molecules against tumors induced by chemical carcinogens, tumors induced by physical carcinogens such as ultraviolet (UV) radiation also seem to be controlled by the immune system (60). Interestingly, UV-induced immune suppression is an important factor in the development of UV-induced tumors, and these tumors are often immunogenic when transplanted into naive hosts but grow in immunosuppressed or $CD8^+$ T cell–depleted mice (61). These data show that immunoediting can also be observed in the UV radiation tumor model as well as the MCA chemical carcinogen model.

Spontaneous Tumor Development in Immunodeficient Mice

An elegant approach to examine the role of the immune system in controlling tumor development is to simply remove specific components of the murine immune system and monitor mice as they age for the development of spontaneous tumors. Mice have long telomeres and display a very low incidence of spontaneous tumor development. For example, we observe incidences of cancer in a variety of inbred wild-type mouse strains that range from 0% to 20% over a two-year period. While many immunodeficient mice also do not develop cancers over a two-year observation period, aging studies have clearly demonstrated a critical role for certain cytotoxic pathways, lymphocyte cellular subsets, and cytokines in the prevention of spontaneous tumor development (**Table 2**). One striking example is the penetrance of immunogenic B cell lymphomas in aged mice (>1 year) on either a C57BL/6 or BALB/c background that increases from 0–6% in wild-type mice to 40–60% in perforin-deficient mice (18, 20). Mice lacking this

STAT: signal transducer and activation of transcription

key T cell and NK cell cytotoxic effector pathway develop an even greater prevalence of B cell lymphomas with an earlier onset when they additionally lack the MHC class I accessory molecule β2-microglobulin (β2m) or IFN-γ compared with perforin alone (20, 62). The absence of other lymphocyte cytotoxic pathways such as TRAIL or FasL also increased the susceptibility of mice to spontaneous lymphomas (63, 64). Collectively, these data provide strong evidence that critical cytotoxic molecules in lymphocytes protect the host from spontaneous tumor development. Intriguingly, human patients with specific mutations in perforin that develop adult onset familial hemophagocytic lymphohistocytosis have recently been identified as also developing leukemia and lymphoma, suggesting the possibility that perforin may protect against hematological malignancies in humans (65).

Aging experiments have also been performed in mice that lack one or more lymphocyte subsets. Although early studies in athymic nude mice did not document an increase in spontaneous tumor development (66), one later study suggested that germ-free nude mice did develop a low frequency of B cell lymphoma compared with heterozygote littermates (67). Unlike other genetic models of immunodeficiency (e.g., SCID mice), the absence of RAG-2 does not affect DNA damage repair pathways in nonimmune cells undergoing transformation. *Helicobacter*-negative 129/Sv *Rag2*$^{-/-}$ mice aged in a specific pathogen-free mouse facility and maintained on broad-spectrum antibiotics developed significantly more spontaneous epithelial tumors (35% gastrointestinal and 15% lung of all mice analyzed at 15–16 months of age) than their wild-type counterparts (17). Consistent with these observations, 129/Sv RAG-2-deficient mice that also lack STAT1 (an important mediator of signaling induced by both type I and type II IFNs) showed an earlier onset and broader spectrum of malignancy, including the development of colon and mammary adenocarcinomas (17). The role of specific lymphocyte subsets in the prevention of spontaneous tumor development has yet to be reported in mice lacking NKT cells, γδ^{+} T cells, or NK cells, but C57BL/6 β2m-deficient mice that lack NKT cells and many CD8^{+} T cells did not have elevated tumor formation upon aging (62), suggesting that distinct lymphocyte populations may play distinct roles, if any, during cancer immunosurveillance of spontaneous tumors.

Similar to chemical carcinogen models of tumor induction, cytokines are critical for the activation of immune effector mechanisms that limit spontaneous tumor development. In one study, a small proportion (<15%) of BALB/c IFN-γ-deficient mice developed lung adenocarcinomas, whereas almost half the IFN-γ-deficient mice on a C57BL/6 background developed a spectrum of various T cell lymphomas, indicating strain-specific differences in the contribution of IFN-γ to prevent spontaneous tumors from occurring (20). In addition, C57BL/6 mice deficient for both perforin and IFN-γ develop more B cell lymphomas with earlier onset than *Pfp*$^{-/-}$ mice, suggesting that in the absence of perforin, IFN-γ can play a role in controlling lymphomas (20). Finally, female mice deficient in the IFN-γ-inducible immunoproteasome subunit LMP2 develop spontaneous uterine neoplasms with a disease prevalence of approximately 36% by 12 months of age (68). This observation suggests that IFN-γ-inducible proteasome function may be essential for MHC class I–mediated tumor rejection.

In addition to the role of cytokines in cancer immunosurveillance of spontaneous tumors, a possible link between tumor immunity and autoimmune or infection-induced inflammation has been raised by several studies. For example, 50% of mice lacking the β2 subunit of the IL-12 receptor (IL-12Rβ2) develop plasmacytomas or lung carcinoma concurrently with immune complex mesangial glomerulonephritis upon aging (69). It is presently unclear why IL-12p40-deficient mice on the same genetic background as the IL-12Rβ2-deficient mice do not display either autoimmunity or spontaneous tumor development (20). Furthermore, mice deficient in both IFN-γ and GM-CSF have been found to develop spontaneous tumors in

a variety of tissues with age, and, in this case, tumor development is associated with acute or chronic inflammatory lesions (70). Maintaining *Gmcsf*$^{-/-}$*Ifng*$^{-/-}$ mice on a regimen of antibiotics delays tumor onset, suggesting that in addition to potentially eliminating tumor cells directly, the immune system might also prevent tumor growth by the timely elimination of infections, thereby limiting inflammation, which is known to facilitate tumor development (71). However, this finding cannot be generalized to all immunodeficient mice that develop spontaneous malignancies because heightened tumor incidence was observed in *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$*Stat1*$^{-/-}$ mice maintained on the same antibiotics regimen (as mentioned above).

Genetic Tumor Models in Immunodeficient Mice

Data supporting the ability of the immune system to suppress tumor development in genetic models of mouse cancer are accumulating rapidly (**Table 3**). Mice heterozygous for the tumor suppressor p53 (*Trp53*$^{+/-}$) are genetically predisposed to tumor development, but in the additional absence of IFNGR1 (14), perforin (18), TRAIL (63), or NKT cells (29), more aggressive tumors develop with an earlier onset, providing strong evidence that these immune components participate in the elimination of nascent transformed cells. More recently, a key role for perforin in immunosurveillance of B cell malignancies has been validated in three different genetic models of B cell malignancies in C57BL/6 mice. Similar to *Pfp*$^{-/-}$*Trp53*$^{+/-}$ mice, perforin-deficient mice that are heterozygous for the tumor-suppressor Mlh1 developed more B cell lymphomas with faster kinetics than mice that are wild-type for perforin and heterozygous for Mlh1 (72). Additionally, perforin protects against the development of oncogene-driven tumors on a transgenic background, including v-abl-driven plasmacytomas and bcl2-driven follicular lymphomas (72).

Other transgenic mice that express oncogenes under the control of tissue-specific promoters have also revealed immune-mediated protection from tumor formation. In one example, IFN-γ suppresses tumor development in mice expressing the human T cell leukemia virus type 1–derived oncogene Tax under the control of a granzyme B promoter (HTLV-Tax transgenic mice) (73). Loss of a single TRAIL-R allele on the lymphoma-prone Eμ-myc genetic background significantly reduced median lymphoma-free survival, corroborating an extrinsic tumor-suppressor role for this cell death pathway (74). The conclusion that NKG2D plays a critical role in cancer immunosurveillance is further supported by the fact that mice defective in NKG2D are more susceptible to Eμ-myc-driven pre-B cell lymphomas (40). Recently, in a study using transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, investigators assessed whether NKG2D controlled the growth of spontaneous oncogene-driven prostate cancer. NKG2D-deficient mice developed more aggressive tumors than wild-type mice; interestingly, these aggressive tumors arising in NKG2D-deficient mice expressed higher amounts of NKG2D ligands than did similar tumors in wild-type mice, suggesting an NKG2D-dependent immunoediting mechanism (40). Also in the same prostate cancer model, the lack of NKT cells in TRAMP *Jα18*$^{-/-}$ mice correlated with more aggressive adenocarcinoma development (75). Despite the presence of a traceable tumor antigen–specific T cell response in these mice, no evidence was found to support a correlation between the presence of NKT cells and the efficacy of cytotoxic T lymphocyte (CTL) responses in this setting. Nevertheless, this study extends the list of spontaneously arising tumors in mice in which NKT cells are critical for natural immune surveillance.

In summary, various cell types including αβ T cells, γδ T cells, NKT cells, and NK cells have been implicated in the processes of elimination and immunoediting, along with a number of effector molecules, including perforin and TRAIL, as well as the cytokines IFN-γ, type I IFNs, and IL-12. More is known about the physiologically relevant targets of IFN-γ's actions than the other

CTL: cytotoxic T lymphocyte

effector molecules or cells during cancer immunosurveillance. Both host and tumor cells are important targets of IFN-γ during the development of protective antitumor immune responses, and the data substantiating this conclusion have already been extensively reviewed (8, 22, 23). The effector cells and cytokines thought to be involved in elimination and immunoediting differ among models, demonstrating that the success of immunoediting and the evidence of its occurrence vary among experimental systems. Indeed, there are models in which the immune system seems to have little influence on the rate of tumor onset or progression (76) and models in which the immune system has a distinct protective role, such as the carcinogen-induced and genetically predisposed tumor models outlined above. The level of immune regulation and tolerance (a state of nonresponsiveness to specific antigens) imparted by the tumors in each of these models might explain, at least in part, why in some cases the effect of subtracting immune elements on tumor progression is less overt. Blocking these tolerance mechanisms might reveal the true mechanisms of tumor-suppressor immunity. Moreover, recent studies have lent great support for the cancer immunoediting hypothesis by validating the existence of the equilibrium phase in multiple models and demonstrating that the immune sculpting actions on tumor immunogenicity occur during this phase.

THE EQUILIBRIUM PHASE: IMMUNE-MEDIATED TUMOR DORMANCY

Historically, tumor dormancy is the term used to describe latent tumors present in patients for decades that may eventually recur as local lesions or form distant metastases (77). Tumors in the equilibrium phase are a subset of dormant tumors that are specifically controlled by components of the immune system. In the equilibrium phase, the host immune system and tumor cells enter a dynamic balance, wherein powerful antitumor immunity contains, but does not fully eradicate, a heterogeneous population of tumor cells, some of which have acquired means of evading immune-mediated recognition and destruction. The equilibrium phase was originally hypothesized to exist to explain the long latency period from the initial transformation event to the escape phase and emergence of malignant disease. In this manner, equilibrium may be the longest of the immunoediting phases where sculpting forces of immunity select for the tumor cells acquiring the most immunoevasive mutations, potentially leading to clinically detectable disease.

Using a low-dose regimen of the carcinogen MCA, we reported the first experimental demonstration that immunity maintains primary occult cancer lesions in an equilibrium state (78). Treatment of naive wild-type mice with low doses of MCA led to overt tumors in only a low proportion of mice. When the remaining carcinogen-treated mice were rendered immunodeficient via depletion of $CD4^+$ and $CD8^+$ T cells and/or neutralization of IFN-γ, sarcomas rapidly grew out at the original carcinogen injection site in approximately 50% of the group. Strikingly, tumor outgrowth was not observed to any significant extent (<10%) in similar MCA-treated wild-type or RAG-2-deficient mice injected weekly with control mAb starting at day 200. Subsequent analyses revealed that mAbs that depleted cells of adaptive immunity (such as $CD4^+$ and $CD8^+$ T cells) or blocked cytokines that promote adaptive immunity (such as IFN-γ and IL-12) caused dormant tumor cells to grow out. In contrast, mAbs that deplete NK cells (anti-NK1.1), block NK cell recognition (anti-NKG2D), or inhibit NK cell effector function (anti-TRAIL) failed to cause the emergence of progressively growing tumors (78). These results support the conclusion that adaptive immunity, but not innate immunity, is responsible for maintaining the equilibrium phase (**Figure 2**). They also help to mechanistically distinguish this phase from elimination, where both innate and adaptive immunity are required. Histological examination of occult tumors revealed the presence of atypical fibroblasts surrounded by a dense infiltration of leukocytes. These atypical

fibroblasts were truly transformed because they formed progressively growing tumors when transplanted into immunodeficient *Rag2*$^{-/-}$ mice. Moreover, occult tumors controlled by immunity displayed fewer Ki67^{+} atypical fibroblasts and more terminal deoxynucleotidyl transferase dUTP end nick labeling (TUNEL) staining cells than progressively growing sarcomas. The visualization of fewer proliferating tumor cells accompanied by more cells undergoing apoptosis is supportive of an active immune response controlling equilibrium tumors. Occult tumors arising after immunodepletion were, on the whole, highly immunogenic, with 40% of the cell lines being rejected after transplantation into wild-type mice. In contrast, the rare spontaneous tumors that grew out of mice treated with control mAbs were poorly immunogenic and grew progressively when transplanted into wild-type recipients. Thus, tumor cells held in equilibrium by adaptive immunity remained highly immunogenic and displayed an unedited phenotype, whereas dormant sarcoma cells that spontaneously escaped immune control to become actively growing tumors displayed reduced immunogenicity, indicating that they had undergone editing.

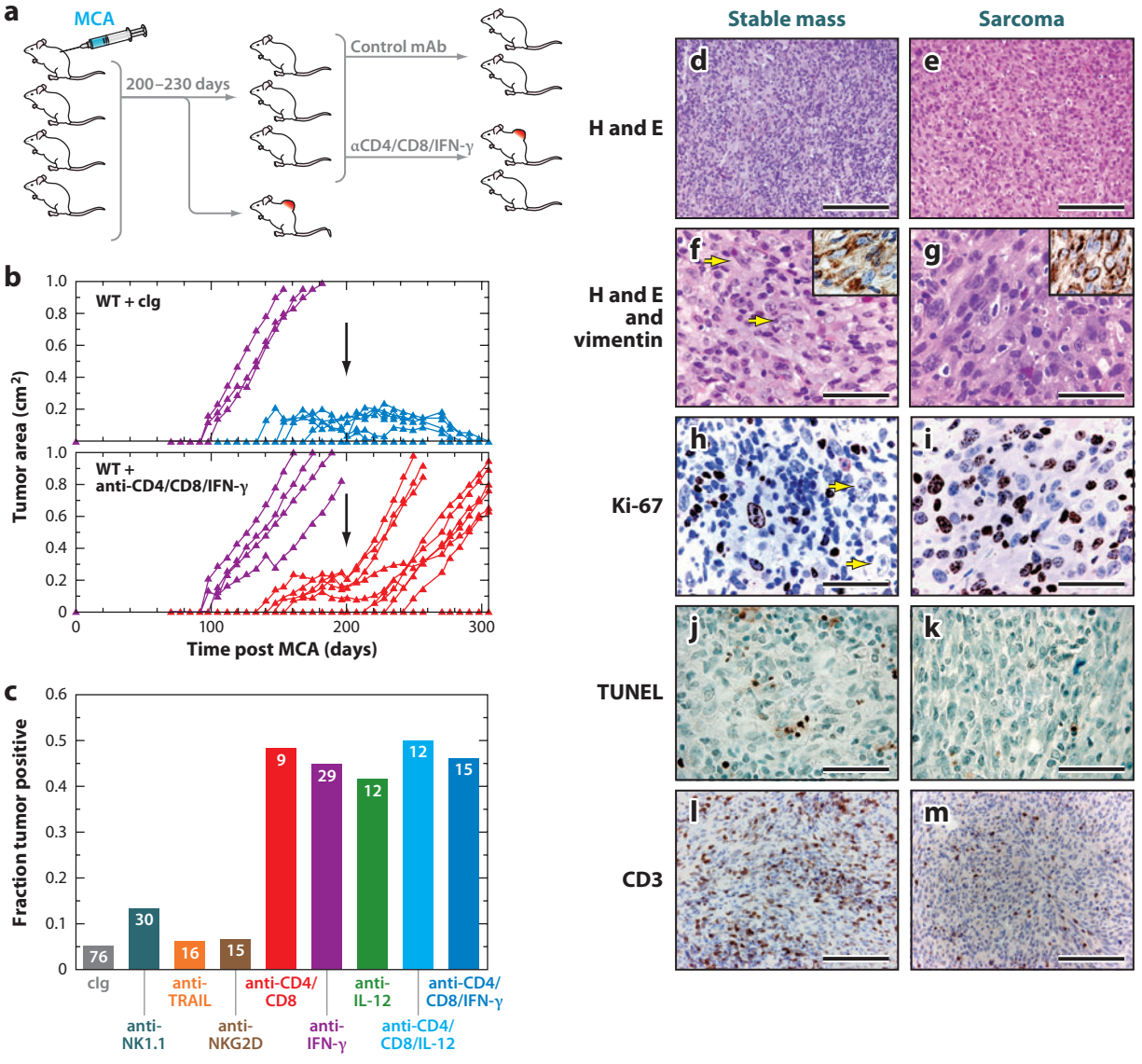
In hindsight, these findings explain previously reported models of immune-mediated tumor dormancy. In the past, most experimental models of tumor dormancy relied heavily on a vaccination-and-challenge strategy with tumor cell lines to induce latent tumor cells. For example, in the BALB/c B cell leukemia/lymphoma 1 (BCL_1) model of tumor dormancy, mice were immunized with BCL_1-derived Ig to create an anti-idiotype vaccine against the B cell receptor (BCR) expressed by the lymphoma cells. Naive, nonimmunized mice injected with BCL_1 tumor cells succumbed to malignancy within 30 days. In contrast, mice initially immunized with BCL_1-derived Ig and subsequently challenged with BCL_1 tumor cells did not develop malignancy, although tumor cells could be detected in the circulation of cancer-free mice hundreds of days after transplantation (79). Over an extended period, vaccinated mice challenged with live BCL_1 tumor cells spontaneously developed malignancy, suggesting an escape from dormancy. Interestingly, when mice harboring dormant BCL_1 tumor cells were depleted of CD8^{+} T cells or IFN-γ using mAbs, the incidence and duration of dormancy were reduced, suggesting that the immune system plays an important role in controlling these dormant tumor cells (79).

Using a BCR-ABL mouse model of leukemia, other investigators also achieved dormancy via a vaccination-and-challenge strategy. The longer the DA1-3b tumor cells remained dormant within the vaccinated host, the greater the expression of programmed cell death 1 ligand 1 (PD-L1) on the tumor cells, which acted to confer resistance to CTL-mediated killing (80). Consistent with the concept of the equilibrium phase, DA1-3b tumor cells acquired advantageous changes over time such that those cells that remained dormant longer were more resistant to attack by CD8^{+} T cells.

Recently, two additional studies using different mouse models of cancer corroborated our findings for the existence of the equilibrium phase by additionally demonstrating that immunity can control primary carcinomas and metastases for extended periods of time. The first study involved a new mouse model of cancer immunosurveillance and equilibrium using ultraviolet B (UVB)-radiation to induce SCCs of the skin. Here, the authors used mice genetically deficient in E3 ligase Casitas B-lineage lymphoma b (Cbl-b), which is known to limit the effector functions of CTLs (81). Thus, mice lacking Cbl-b exposed to UVB-radiation developed fewer spontaneous SCCs compared with wild-type mice due to the enhanced antitumor activity of CD8^{+} T cells that lack the Cbl-b regulator. *Cblb*$^{-/-}$ mice that failed to form carcinomas 400 days after UVB treatment were then divided into two experimental groups. One group received mAbs that depleted CD8^{+} T cells, whereas the other group received control mAbs. Only 10 days after starting mAb treatment, nearly 50% of mice depleted of CD8^{+} T cells developed rapidly growing tumors, whereas none of the mice receiving

control mAb developed detectable tumors (81). It will be interesting to determine in the future whether wild-type $CD8^+$ T cells can also maintain occult UVB-induced carcinomas in an equilibrium state.

A second study demonstrates that immunity can prevent the outgrowth of micrometastases for an extended period of time in an oncogene-driven model of melanoma. In this model, transgenic mice that express the human *RET* oncogene and a chimeric mouse/human MHC antigen (AAD) specifically in melanocytes were found to develop extensive disseminated metastases (82). Depletion of $CD8^+$ T cells in RET AAD mice significantly accelerated the outgrowth of metastatic lesions to visceral organs, indicating that immunity is one significant barrier disseminated tumor cells must overcome to establish metastatic disease (82). Interestingly, these $CD8^+$ T cells did



not seem to directly kill the tumor cells, but rather mediated cytostatic effects on the disseminated tumor cells. One likely mechanism underlying control of disseminated tumor cell outgrowth may be via IFN-γ produced by tumor antigen-specific T cells, which has been shown in other systems to inhibit cellular proliferation and curtail angiogenesis (83–85). For example, in a preclinical model of pancreatic cancer using RIP-Tag2 mice, the transfer of IFN-γ-producing TNFR1$^+$ CD4$^+$ T cells specific for Tag prevented the progression of pancreatic islet cancer (85). In this study, transferred Tag-specific T cells arrested tumor cell proliferation and prevented angiogenesis, curtailing tumor growth and resulting in the inhibition of multistage carcinogenesis and induction of a period of extended tumor dormancy. In the absence of either TNFR1 signaling or IFN-γ receptor signaling, the same T cells paradoxically promoted angiogenesis and multistage carcinogenesis. Currently, the adoptive transfer of cancer-reactive T cells into human cancer patients is an experimental therapy with promising results (86), and it will be interesting to see if these therapies can be optimized as a viable therapeutic endpoint to induce an equilibrium state in some patients.

THE ESCAPE PHASE: FAILURE OF CANCER IMMUNOSURVEILLANCE

Tag: SV40 large T antigen

Although the processes of cancer elimination and equilibrium largely occur behind the scenes, a more dramatic result of cancer immunoediting can occur when tumors escape immune control, leading to the appearance of overt cancer (**Figure 3**). Thus, the escape phase represents the failure of the immune system either to eliminate or to control transformed cells, allowing surviving tumor cell variants to grow in an immunologically unrestricted manner. Cancer cells undergoing stochastic genetic and epigenetic changes generate the critical modifications necessary to circumvent both innate and adaptive immunological defenses. Moreover, the immune system contributes to tumor progression by selecting more aggressive tumor variants, suppressing the antitumor immune response, or promoting tumor cell proliferation. The interaction between a heterogeneous population of cancer cells undergoing rapid genetic modifications and the constant immunological pressure exerted by immune cells allows for the Darwinian selection of the most fit tumor variants to survive and form overt cancer in immunocompetent hosts. Thus, nearly all human cancers and

Figure 2

Experimental evidence for the existence of the equilibrium phase of cancer immunoediting. (*a*) Protocol to test for the existence of occult tumors held in an equilibrium state. (*b*) Sixteen wild-type C57BL/6 mice were injected with 25 μg of MCA and monitored for tumor formation. At 200 days, the remaining 13 tumor-free mice were treated weekly with control immunoglobulin (cIg) and monitored for the appearance of tumors (*upper panel*). Nineteen wild-type C57BL/6 mice were similarly injected with MCA and at 200 days, the remaining 15 tumor-free mice were treated weekly with a combination of anti-CD4/CD8/IFN-γ (*lower panel*). (*c*) Summary of MCA equilibrium experiments showing the fraction of MCA-treated mice developing late-forming tumors after treatment with antibodies starting at day 200. Only when MCA-treated mice are administered antibodies targeting components of adaptive immunity (IL-12, IFN-γ, and CD4$^+$ and CD8$^+$ T cells), but not innate immunity (NK1.1, TRAIL, and NKG2D), are tumors held in an equilibrium state free to expand. (*d*, *f*, *h*, *j*, *l*) Histological sections from representative stable masses and (*e*, *g*, *i*, *k*, *m*) progressively growing MCA sarcomas isolated from MCA-treated 129/SvEv mice. Both stable masses and sarcomas have atypical cells (*yellow arrows*) that can be visualized by H&E staining and stained for the fibroblast marker vimentin (*inset of f and g*). Stable masses display less staining for the proliferation marker Ki-67 (*h*), more staining for the apoptotic marker TUNEL (*j*), and more staining for infiltrating CD3$^+$ immune cells (*l*) than progressively growing MCA sarcomas (*i*, *k*, *m*). The visualization of fewer proliferating cells accompanied by more cells undergoing apoptosis is supportive of an active immune response controlling equilibrium tumors. Magnification: 200×, scale bars 100 μm (*d*, *e*, and *l*); 400×, scale bar 50 μm (*m*); 600×, scale bars 33 μm (*f–i*); 1,000×, scale bars 20 μm (*j* and *k*). (Abbreviations: H&E, hemotoxylin and esoin; MCA, 3′-methylcholanthrene; TUNEL, terminal deoxynucleotidyl transferase dUTP end nick labeling.) This figure is reprinted and modified, with permission, from *Nature* (78).

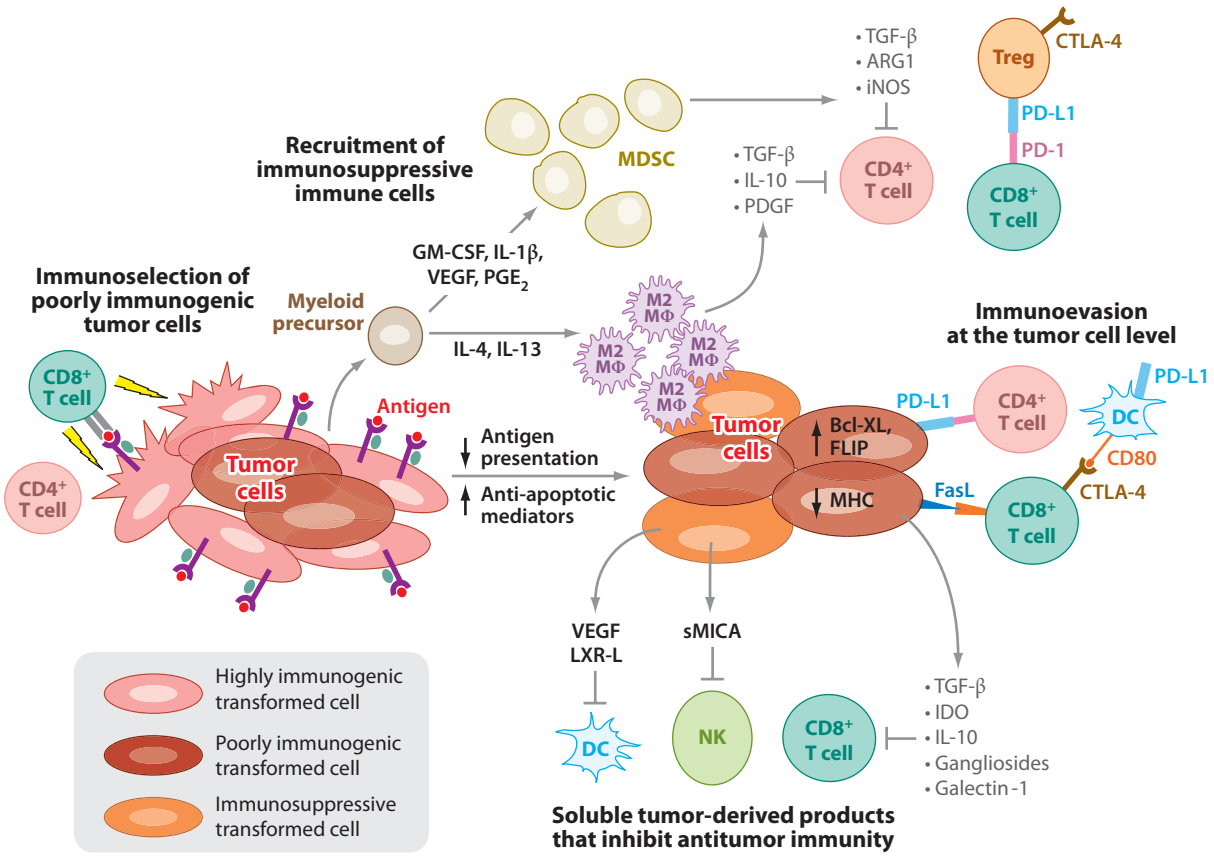


Figure 3

Tumor escape mechanisms. The immune system exerts selective pressure on tumors through a variety of processes, including the destruction of antigen-positive tumor cells by $CD8^+$ T cells. As a result, immunogenic tumor cells are eliminated, leaving behind tumor cell variants more adept at evading immune-mediated destruction (i.e., immunoselection). Over time, tumors evolve mechanisms to elude or inhibit immunity by both intrinsic and extrinsic means. Intrinsic alterations within tumor cells evade immunity by downregulating antigen presentation (MHC), upregulating inhibitors of apoptosis (Bcl-XL, FLIP), or expressing inhibitory cell surface molecules that directly kill cytotoxic T cells (PD-L1, FasL). In addition, tumor cells secrete factors that inhibit effector immune cell functions (TGF-β, IL-10, VEGF, LXR-L, IDO, gangliosides, or soluble MICA) or recruit regulatory cells to generate an immunosuppressive microenvironment (IL-4, IL-13, GM-CSF, IL-1β, VEGF, or PGE_2). Once recruited, regulatory cells attenuate antitumor immunity through the liberation of immunosuppressive cytokines and alterations in the nutrient content of the microenvironment. Specifically, secretion of IL-4 and IL-13 leads to recruitment and polarization of M2 macrophages (M2 MΦ) from myeloid precursors, which express TGF-β, IL-10, and PDGF that inhibit T cells. The release of colony-stimulating factors, IL-1β, VEGF, or PGE_2 by tumor cells results in the accumulation of MDSCs that can block T cell function by expressing TGF-β, ARG1, and iNOS. Regulatory T cells (Tregs) can also inhibit effector T cells through multiple mechanisms, including expression of CTLA-4. (Abbreviations: ARG1, arginase 1; Bcl-XL, B cell lymphoma extra long; CTLA-4, cytotoxic T lymphocyte associated protein-4; DC, dendritic cell; FasL, Fas ligand; FLIP, apoptosis-stimulating fragment-associated protein with death domain-like interleukin-1 converting enzyme-like inhibitory protein; GM-CSF, granulocyte macrophage colony–stimulating factor; IDO, indoleamine 2,3-deoxygenase; IL, interleukin; iNOS, inducible nitric oxide synthase; LXR-L, liver X receptor ligand; MDSC, myeloid-derived suppressor cells; MHC, major histocompatibility complex; MICA, MHC class I polypeptide-related sequence A; PDGF, platelet-derived growth factor; PD-L1, programmed cell death 1 ligand 1; PGE_2, prostaglandin-E_2; TGF-β, transforming growth factor-β; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.)

experimental cancer cell lines are those that have evaded immunological control. The focus of this section on tumor escape is not to provide an exhaustive list of escape mechanisms that have been extensively reviewed elsewhere (8, 9, 22, 23, 25, 87), but rather to shape the framework of how tumor cells achieve immunological escape. Although the mechanisms for tumor escape are varied, they can be categorized generally as cell-autonomous modifications at the level of the tumor cell that directly evade immune detection and destruction or modifications in immune cells effected by tumor cells to generate an immunosuppressive network.

Tumor Cell Modifications to Evade Immune Detection or Destruction

Tumor escape can result from changes that occur at the level of the tumor by directly inhibiting tumor recognition or cytolysis by immune effector cells. In some cases, immune evasion by tumors is absolute and the immune system has little impact on tumor progression, whereas in other cases tumor growth is delayed before the immune system is overwhelmed, leading to tumor progression. In its simplest form of escape, tumor cells can evade detection due either to a lack of immunological recognition or to the induction of central or peripheral tolerance. Central tolerance is a process whereby self-reactive T cells are eliminated or converted to a regulatory phenotype in the thymus (88). In this case, and in the absence of neoantigen expression, tumors may remain invisible to the adaptive immune system and are free to grow unhindered. Peripheral tolerance is an important process whereby T cells reactive with self-antigens not expressed in the thymus are deleted or rendered nonresponsive in the periphery. In this case, some level of antitumor immune response may be initiated transiently before tolerance is induced, leading to tumor progression (76, 89).

In addition to tolerance induction, tumor cells can acquire defects in antigen processing and presentation pathways that facilitate evasion from adaptive immune recognition. Specifically, loss of TAP1, MHC class I molecules, β2m, LMP2, and LMP7 and the development of IFN-γ or IFN-α/β insensitivity by tumor cells prevents T cell–mediated elimination, resulting in tumor progression (24, 90–92). An extreme version of this escape process occurs when tumors lose the ability to respond to IFN-γ through either mutation or epigenetic silencing of genes encoding the IFN-γ receptor signaling components (IFNGR1, IFNGR2, JAK1, JAK2, and STAT1) (93). In this case, the affected tumor cells not only fail to upregulate MHC class I proteins but also are unable to produce the intracellular machinery that facilitates antigen processing and presentation (i.e., TAP1, TAP2, and components of the immunoproteasome). In addition, genomic instability within tumor cells may result in the loss of TSAs, creating antigen loss variants that are no longer detectable by antigen-specific $CD8^+$ T cells. Similarly, tumors can become unrecognizable to cells of the innate immune system through loss of ligands for the NK cell effector molecule NKG2D (94) or through suppression of the production of proinflammatory danger signals to impair dendritic cell (DC) maturation (95). Thus, tumor cells may avoid recognition by adaptive or innate immune cells by multiple mechanisms.

Additionally, tumor cells that are unable to avoid immune cell detection may develop mechanisms to evade immune-mediated killing. Even when antigens continue to be expressed, tumors can evade effector lymphocytes by upregulating expression of antiapoptotic molecules such as FLIP and BCL-XL (96, 97). Alternatively, resistance to lysis by immune cells can be acquired through expression by tumors of mutated inactive forms of death receptors, including the TRAIL receptor, DR5 (98), and Fas (99).

The above strategies of immune escape can be viewed as passive, involving the loss of recognition or reduced sensitivity to apoptosis. However, tumor cells can take a more active and direct role in subduing immunity through expression of immune-inhibitory ligands on their surface that inhibit the cytotoxic actions

JAK: janus-activated kinase

DC: dendritic cell

of immune cells after tumor cell recognition in a cell-contact mediated manner. The expression of B7-H1 (PD-L1) (100), HLA-G (101), and HLA-E (102) on the cell surface of tumor cells interacts with receptors on the cell surface of T cells to dampen the cytotoxic actions of T cells or induce apoptosis within the T cell. In addition, tumor cell expression of HLA-E or HLA-G can modify the actions of innate immune cells by inducing tolerance in antigen-presenting cells and inhibiting NK cell–mediated killing (101). The actions of tumor cells to impede the development of antitumor immune responses are not limited to changes that occur directly at the level of the tumor, but also result from the elaboration of cytokines and molecules that act at a distance to generate an extensive immunosuppressive network that facilitates tumor progression.

Generating an Immunosuppressive Tumor Microenvironment

The development of an immunosuppressive environment concomitantly with tumor development is evidenced by observations in which protective responses against transplantable tumors can be generated when immunotherapies are delivered prior to tumor challenge but fail against established tumors (103). Importantly, local immunosuppression in the tumor microenvironment seems to cause failure of therapy against established tumors because tumor-bearing mice can often respond normally to independently administered antigens (104). The development of an immunosuppressive state is achieved by tumor cells that inhibit the function of effector immune cells or recruit the efforts of regulatory immune cells to evade immunological elimination in a paracrine or endocrine manner.

Tumor cells secrete factors to directly inhibit the function of sentinel immune cells of both the innate and adaptive arms of immunity. For example, tumor cells can block T cell and NK cell function through secretion of soluble forms of ligands for effector molecules, as has been reported for shed ligands of NKG2D (105). In addition, antitumor immunity can be subverted at an early stage by tumor-derived factors that inhibit DC function. In response to danger signals and cellular stress, DCs are stimulated to mature, migrate, and carry tumor antigens to lymph nodes to alert the adaptive arm of immunity to the presence of transformed cells. To inhibit this initial immune priming event, tumor cells secrete sterol metabolites to suppress the expression of CCR7 on the cell surface of DCs, thereby disrupting DC migration to the lymph nodes (106). A recent study demonstrates that unknown tumor-derived factors induce the expression of scavenger receptor A on DCs, resulting in excessive uptake of extracellular lipids that reduces their capacity to process antigens (107). Furthermore, many tumors produce vascular endothelial growth factor (VEGF), which is critical for the establishment of one of the hallmarks of cancer development, angiogenesis, but also prevents endogenous DC function. Targeted monoclonal antibodies against VEGF improve DC function in vivo and improve the efficacy of cancer immunotherapies (108).

Simultaneous inhibition of multiple stages in the development of antitumor immunity can be achieved through the liberation of immunosuppressive cytokines by tumor cells. For example, TGF-β secretion by tumor cells leads to inhibition of DC activation as well as direct inhibition of T cell and NK cell function (109). Similarly, IL-10 present within tumors can suppress DC function and skew T cell responses toward a type 2 immune response that is less effective against malignant cells (110). However, the role of IL-10 in tumor immunity remains somewhat obscure because it has been shown also to enhance immune destruction of tumors (111).

Other tumor-derived factors can be more selective in inhibiting particular components of immune responses but can still effectively suppress immunity. For example, production of galectin can impede T cell activity and survival, and blocking this factor can aid tumor rejection in mice (112). In addition to using cytokines and lectins to downregulate immune

responses, tumors can secrete enzymes that metabolize amino acids within the tumor microenvironment. Specifically, expression of IDO by tumor cells metabolizes tryptophan to generate kynurenines and inhibits $CD8^+$ T cell proliferation and promotes $CD4^+$ T cell apoptosis (113). Two potential mechanisms of immune inhibition include starvation, where depletion of this important amino acid weakens T cells, and metabolite cytotoxicity, where metabolic products of tryptophan degradation inhibit T cell function (114).

In addition to the mechanism described above, a variety of immunosuppressive regulatory leukocytes can suppress immune function, leading to tumor escape. Regulatory T cells (Tregs), largely expressing CD4, CD25, and Foxp3, inhibit CTL function in a number of ways, including IL-10 and TGF-β production, CTLA-4 and PD-L1 expression, and IL-2 consumption (115). This regulatory lymphocyte is the critical mediator of peripheral tolerance under physiological settings but is often recruited to the tumor site where it suppresses antitumor immunity. Furthermore, TGF-β production by tumor cells can convert effector T cells into Tregs that, in turn, suppress other effector T cells, which infiltrate the tumor mass (116). Experimental tumor models that eliminate Tregs result in robust antitumor immune responses and the rejection of transplanted or primary tumors (117). In addition to Tregs, other regulatory lymphocyte populations can be found in subsets of NKT cells and B cells that inhibit effector responses against transformed cells (118, 119).

The production and elaboration of GM-CSF, IL-1β, VEGF, and PGE_2 by tumors lead to expansion of myeloid-derived suppressor cells (MDSCs) and their accumulation within the tumor mass (120). MDSCs are a heterogeneous group of myeloid progenitor cells and immature myeloid cells that can inhibit lymphocyte function by a number of mechanisms. These include the production of immunosuppressive cytokines (TGF-β) (121); the depletion or sequestration of amino acids arginine or cysteine, which are required for T cell function (122); the inhibition of T cell activation by TCR nitrosylation (123); and the induction of Tregs (124). The multiplicity of mechanisms that inhibit lymphocytes in either an antigen-specific or antigen-nonspecific manner most likely reflects distinct cellular subsets within the MDSC heterogeneous population (125).

In addition to MDSCs, plasmacytoid DCs (pDCs) are recruited to the tumor mass and become key players in the immunosuppressive network. Ovarian cancer cell products activate pDCs, which in turn induce the expansion of IL-10-producing $CD8^+$ Tregs (126). A potentially novel subset of DCs, sometimes referred to as vascular leukocyte cells (VLCs) or $Tie2^+$ monocytes, are recruited to the tumor bed by β-defensins and induce their endothelial-like specialization, where they enhance vasculogenesis and suppress conventional DC function through the secretion of VEGF and other proinflammatory cytokines (127). A recent study by Shields et al. (128) identified lymphoid tissue-inducer (LTi) cells that are recruited by CCL21-secreting melanomas and contribute to the development of an immunosuppressive tertiary lymphoid structure within the tumor mass that recruits MDSCs and Tregs and polarizes monocytes to M2 macrophages. Many tumors attract tumor-associated macrophages (TAMs) by IL-4 and IL-13. M2 macrophages can inhibit antitumor immunity through the production of TGF-β and IL-10 and can promote stromal development and angiogenesis through secretion of platelet-derived growth factor (PDGF) (129).

Together, these examples demonstrate that, in addition to central and peripheral tolerance, failure of antitumor immunity can be due to the development of an immunosuppressive microenvironment. Any one, or a combination of several, of the above cellular and molecular mechanisms can contribute to suppression of tumor immunity. The balance between these inhibitory mechanisms and immune-stimulating conditions determines whether or not tumors escape immune responses and the rate of tumor progression. In human cancer patients, immunosuppression of lymphocytes

within the tumor microenvironment has also been widely observed for a variety of cancer types (130). In the next section, we discuss the evidence for cancer immunoediting in humans, with particular emphasis on the elimination and equilibrium phases.

CANCER IMMUNOEDITING IN HUMANS

The extensive studies reviewed above clearly demonstrate that the immune system not only protects against tumor development but also shapes tumor immunogenicity in mouse models of cancer. The question therefore naturally arises whether cancer immunoediting occurs in humans. Humans are not clean models of immune deficiency such as those that exist in experimental mice that live in controlled environments. Nevertheless, compelling clinical data support the existence of cancer immunoediting in humans. Here, we do not discuss the mechanisms of tumor escape in humans because they greatly overlap with those observed in mice (discussed above) and have been extensively reviewed elsewhere (8, 9, 22, 23, 25, 87), but rather we review data supporting an active immune response eliminating or controlling cancer in humans.

Acquired Immunodeficiency and Cancer Risk

Although severely immunodeficient humans succumb to infections relatively early, advances in the management of acquired immunodeficiencies have led to extended survival of patients with partly compromised immune systems. Evidence for immunosurveillance can be found in patients with AIDS who have an increased frequency of malignancies (131). Most often, these malignancies are virus-associated and initiated by viral oncogenes, including lymphomas (Epstein-Barr virus), Kaposi's sarcoma (herpesviruses), and urogenital cancers such as cervical cancer (human papilloma viruses) (132). Although the antigenic targets of the above malignancies are not fully characterized, viral antigens can certainly be expressed, and an argument can be made that the increased frequency of virus-associated cancers reflects a breakdown in antiviral immunity rather than reduced immunosurveillance of cancer. However, support for immunosurveillance can be found in malignancies of nonviral origin.

The incidence of nonvirally induced tumors in AIDS patients is less well documented, but there is evidence of an increased incidence of solid cancers in AIDS patients, particularly lung adenocarcinomas (133). Although a large proportion of HIV-infected individuals may be exposed to other lifestyle risk factors, including smoking, the association of lung cancer in AIDS patients has been demonstrated to be independent of smoking (134), with a 3.5-fold elevated risk of lung cancer for AIDS patients compared with the wider population.

Immunosuppressed Organ Transplant Recipients and Cancer Risk

Some level of immunodeficiency can be induced in humans by the use of immunosuppressants following organ transplantation, and an increase in the incidence of malignancies in these patients suggests a role for immunosurveillance in humans. Greater cancer prevalence among transplant recipients has been observed in a range of transplant situations using a variety of immunosuppressants. For example, patients receiving kidney transplants display a threefold increase over the general population in the overall incidence of malignancy. Although virus-associated malignancies predominate, there is also an increased risk for developing noninfectious cancers of the colon, lung, pancreas, kidney, and endocrine system (135). Additionally, a dramatic increase in risk (200-fold) of nonmelanoma skin cancers has been demonstrated in renal transplant patients, suggesting a particularly important role for cancer immunosurveillance at sites exposed to UV irradiation (136). Finally, melanomas have also been observed to increase in frequency in these renal transplant patients, but to a lesser degree (two- to tenfold) than other skin cancers (136, 137).

In addition to renal transplant recipients, patients that have received heart transplants and undergone immunosuppression to facilitate organ engraftment have a reported 2.7-fold increased risk of cancer development over the general population. However, the increased risk varied between malignancies, with non-Hodgkin's lymphoma predominating at a 22.7-fold increased risk (138) and lung cancer incidences that varied from 2- to 25-fold increased risk (138, 139). Patients with liver transplants also manifest a greater preponderance of cancers not associated with pathogens, including some types of head and neck cancers and nonmelanoma skin cancers (140, 141). Furthermore, the positive correlation between the duration of pharmacology-induced immunosuppression and the incidence of cancer development also supports the existence of human cancer immunosurveillance (142).

Additionally, cancer risk in transplant recipients varies with the type of immunosuppressive regimen utilized, where risk of nonmelanoma skin cancer is different between patients receiving mycophenolate (143) versus antibody induction therapy (141). Interestingly, incidences of some cancers, including breast, prostate, ovarian, brain, and testicular, have not been observed to increase in the context of pharmacologically induced immunosuppression, but it is not clear if these malignancies are less immunogenic or simply take longer to develop. These data support the notion that de novo malignancies arise due to the permissive environment created by immunosuppressive regimens, which inhibit cancer immunosurveillance mechanisms. Further supporting the link between immunosuppression and malignancy are observations of spontaneous remissions of lymphomas after cessation of immunosuppression (144).

Spontaneous Immune Responses to Cancer

The spontaneous recognition and destruction of human cancers by cells of the adaptive immune system substantiate the occurrence of cancer immunosurveillance in humans. As early as the 1970s, screening cancer cell lines with autologous patient serum identified spontaneous antibody responses to autologous cancers in a subset of patients (145, 146). Antibody responses in patient serum have been reported for more than 100 tumor-associated antigens (TAAs), although only eight antigens have been identified in multiple reports, suggesting that many immunogenic mutations might be unique for each individual cancer (reviewed in Reference 147). Among the shared antibody responses were those against the cancer-testis antigen NY-ESO-1 and the mutant forms of tumor suppressor p53, which are often overexpressed in many different types of human malignancies (148, 149). The high frequency of antibodies specific for TAAs in cancer patients compared with healthy individuals suggests that immunity has been induced in response to malignancy. The reasons for spontaneous antibody responses in cancer patients are not known but may include an overabundance of antigen or its enhanced presentation to generate immunogenicity in the malignant setting.

TAA: tumor-associated antigen

The phenomenon of spontaneously regressing melanoma lesions accompanied by the clonal expansion of T cells is arguably the strongest evidence for the elimination phase of cancer immunoediting in humans (150–152). These responses, observed in the absence of specific immunotherapy, support the ability of the immune system to spontaneously recognize antigens on/in tumors. Specific $CD4^+$ and $CD8^+$ T cell activity against TAAs, including NY-ESO-1, is known to develop spontaneously in human cancer patients (153, 154). However, spontaneous T cell responses specific for some TAAs, such as the MAGE family, are rare (155), whereas those specific for the melanocyte differentiation antigen MART-1/Melan-A have been found in a relatively high percentage (>50%) of healthy individuals (156). Thus, there is a strong correlation between spontaneous T cell responses and some TAAs but not others, and it is not clear whether the presence of TAA-specific T cells in healthy individuals reflects past exposure

TIL: tumor-infiltrating lymphocyte

to transformed cells expressing the antigen. More studies are needed to identify TAAs and TSAs in a variety of cancers to determine the relative abundance and uniqueness of tumor antigens.

Other spontaneous immune responses against malignant cells have been demonstrated in patients with paraneoplastic autoimmune disorders (PND) caused by cross-reactivity between the antitumor immune response and neurologic antigens (157). In addition to antibody responses, tumor-specific T cells have also been identified in patients with PND (158). Nearly all patients with PND die from cancer or neurologic disease; however, the few surviving patients have complete tumor remission in response to therapy and no longer manifest any neurological impairment. These dramatic clinical cases demonstrate that the tumor antigens are the drivers of both beneficial immune responses against neoplastic tissues and pathological immune responses against normal tissues (i.e., neurons). Interestingly, PND symptoms can precede tumor diagnosis by several years (159), indicating that antitumor responses might be primed by undetectable, microscopic tumors early in their evolution. It remains to be determined whether the antitumor immune response substantially delays tumor growth in patients with PND, and such analysis is likely to be confounded by the lethality of the neurologic complications. Nevertheless, the presence of antineuronal antibodies has been reported to correlate with improved prognosis at least for some neurological malignancies (160), and there are some case reports of spontaneous complete remission in the absence of specific treatment (161). Spontaneous tumor regression accompanied by lymphocyte infiltration has also been noted for a number of other tumor types (reviewed in Reference 26); however, the role of lymphocyte infiltration in tumor regression has not been established in these cases due to their rarity. Even in the absence of spontaneous tumor regression, tumor-infiltrating lymphocytes (TILs) appear to be controlling tumor outgrowth and enhancing patient survival, as discussed below.

Tumor-Infiltrating Lymphocytes as a Prognostic Indicator

Further support for cancer immunoediting can be found in reports that correlate the frequency of TILs with patient survival. Tumor infiltration by T cells, NK cells, or NKT cells has been associated with an improved prognosis for a number of different tumor types (162–169). The initial association between favorable patient prognosis and TILs was first observed in patients with melanoma (162, 163), where it was reported that patients with high levels of $CD8^+$ T cell infiltration survive longer than those whose tumors contain low numbers of lymphocytes. Since then, various melanoma-specific antigens have been identified in addition to melanoma-specific T cells in patients with melanoma (reviewed in Reference 170).

In a landmark study in ovarian cancer, the presence of TILs in ovarian cancer tissue specimens correlated with better prognosis. Specifically, 38% of patients with high numbers of TILs survived more than five years after diagnosis compared with 4.5% of patients with low numbers of TILs (165). These findings have been confirmed in subsequent studies for ovarian cancer (166) and for other malignancies, including melanoma (171) and colon cancer (167–169). Particularly elegant studies on colon and lung cancers reveal a tight correlation between the quality and quantity of intratumor immune responses and patient survival (168, 169). Remarkably, the type and density of lymphocytes infiltrating these cancers were more powerful prognostic indicators than previous pathological criteria for tumor staging, underscoring the need for clinical pathologists to consider infiltrating immune cells when determining a patient's prognosis. In fact, the results of these studies provide strong evidence for the equilibrium phase of cancer immunoediting in humans. Enhanced survival of some cancer patients is

associated with particular subsets of T cells, such as the intratumor localization of $CD8^+$ T cells and Tregs (164, 166, 167). However, tumor infiltration by some leukocytes, such as macrophages and Tregs, sometimes has a detrimental impact on patient survival (172). A particularly interesting disagreement concerns the significance of Tregs in tumors, where some groups find a correlation between the presence of $Foxp3^+$ Tregs in tumors with a poor prognosis (173, 174), whereas other groups report better prognosis if Tregs are present in tumor tissue samples (175, 176). Reasons for these different outcomes are not clear but may be related to the type of malignancy involved.

Immunogenicity of Cancers with Microsatellite Instability

All cancers are inherently genetically unstable, and this instability seems to be a contributing factor in the capacity of immune cells to detect and control tumor cells. For example, the infiltration of colorectal cancer by $CD8^+$ T cells is associated with a favorable prognosis (167–169), as discussed above, and this association is further strengthened in cases where tumors exhibit high levels of a particular type of genetic instability, referred to as microsatellite instability (MSI), where defects in DNA mismatch repair mechanisms lead to the duplication or deletion of short repeated sequences of DNA known as microsatellites (177, 178). Strikingly, MSI-high (MSI-H) tumors are often strongly infiltrated with lymphocytes, including activated $CD8^+$ T cells (178), and contain tertiary lymphoid follicles (177) indicative of a potent local immune response. The high rate of mutation in MSI-H tumors has been shown to result in the generation of a number of novel tumor antigens that can be recognized by B cells, $CD4^+$ T cells, and $CD8^+$ T cells. Together, these findings suggest that the generation of antigenic peptides as a result of genomic instability might result in the priming of a protective adaptive immune response in patients with MSI-H colorectal cancers. An interesting possibility is that these findings are not unique to colorectal cancers, but apply to other human cancers as well (179).

Cancer Equilibrium in Human Patients

A plethora of clinical evidence suggests that occult cancers can lie dormant in patients for many years, sometimes exceeding 20 years, before malignant disease progresses to clinically detectable levels (180). For example, 20–45% of patients with breast or prostate cancer will relapse years or even decades later (181, 182). Such a lengthy and protracted period from initial cancer remission to cancer recurrence may, in part, be explained by immunological constraints placed on the remaining cancer cells. In some cases, circulating disseminated cancer cells exist for decades after treatment without the reestablishment of clinical disease from these persistent cancer cells (183). This is known as minimal residual disease, and it appears to be a common reservoir of cancer cells for most cancer types after the initial therapeutic intervention, but its mechanisms for maintenance are poorly understood. Minimal residual disease is of critical importance because the vast majority of morbidity and mortality associated with cancer is due to metastatic lesions that are presumed to be seeded by these persistent cancer cells. There is evidence that immunosuppressive intervention for various conditions can be associated with a greatly increased risk of cancer relapse even after long periods of time. In one study, three out of eight patients (37%) experienced cancer relapse following immunosuppression after more than 10 years of remission, whereas cancer patients in remission for 10 years or more that had not undergone immunosuppressive treatment had only a 2% relapse rate (180).

One remarkable clinical scenario that suggests that immunity can prevent the outgrowth of occult lesions is the unintentional transplantation of cancer cells from organ donor to immunosuppressed recipient. In these

scenarios, organs were harvested from deceased donors, who either had no previous clinical history of malignancy or were in cancer remission and had no overt signs of disease at the time of organ donation and transplantation into recipients. The recipient patients undergoing immunosuppression for organ engraftment later developed clinically detectable cancers of donor origin (184, 185). A subset of these donor-derived malignancies was from donors with no previous history of cancer, suggesting a state of equilibrium operating between cancer cells in the primary lesion and the donor's immune system that subsequently broke down after transplantation into immunosuppressed recipients.

Clinicians have long observed that the immune system mounts a response against preneoplastic cells in monoclonal gammopathy of unknown significance (MGUS) but does not eliminate them, eventually allowing MGUS to progress to multiple myeloma (186). The ability to detect this premalignant phase of disease allows for immunologic monitoring throughout disease progression, and such monitoring has revealed that T cells derived from the bone marrow of patients with MGUS mount strong responses to autologous premalignant cells, but these responses are absent in patients with multiple myeloma (187). These findings are consistent with the idea that T cells may hold premalignant cells in check for an extended period of time (i.e., equilibrium) but eventually fail to control some abnormal plasma cell clones that ultimately give rise to multiple myeloma (i.e., escape). Additionally, treatment of low-grade B cell lymphoma by administering antibodies specific for the idiotype expressed by the malignant cells results in remission of disease without entirely eliminating the tumor cells, and these circulating lymphoma cells are detected up to eight years after treatment without any other signs of progressive disease (188). These results suggest that equilibrium may be a viable therapeutic endpoint for the treatment of cancer, and in such a case, interventions may be necessary to stabilize the equilibrium phase indefinitely and prevent the immunoselection of tumor cell variants possessing novel mutations that eventuate in resistance to immune attack.

Summary of Human Cancer Immunoediting

As discussed above, there is considerable clinical evidence for the cancer immunoediting process in humans even though cancer patients are a genetically and immunologically diverse population. The confluence of these complex factors may explain why spontaneous immune responses occur in only a proportion of individuals and why some patients respond better to certain immunotherapies. The differences in an individual's immune repertoire, the capacity to process and present antigens, the quality and quantity of tumor antigens generated, as well as the ability of cancer to suppress antitumor immunity help to determine the overall outcome. Future advances in gene expression and proteomics of human cancers and their antigens will provide greater insight into the mechanism of cancer immunoediting in humans, which may be critical in determining which patients benefit from particular treatments.

CANCER-RELATED INFLAMMATION AND CANCER IMMUNOEDITING: INTERDEPENDENT PROCESSES

Inflammation is a broad and complex physiological process that maintains tissue homeostasis in response to tissue stressors such as infection or tissue damage (189). Rudolph Virchow, who established the cellular basis of pathology, was the first to propose the link between inflammation and cancer in the 1860s when he observed leukocytes infiltrating neoplastic tissues (190). We now appreciate that chronic inflammation can contribute to cancer initiation by generating genotoxic stress, cancer promotion by inducing cellular proliferation, and cancer progression by enhancing angiogenesis and tissue invasion. However, there is overwhelming evidence that immunity against transformed cells can develop to protect

the host from cancer formation, as discussed above. Each of the six cell-intrinsic hallmarks of cancer can influence the immune system (9), and the cancer immunoediting process attempts to describe the varied outcomes of tumor–immune system interactions, including immunosurveillance (antitumor), immunoselection (protumor), and immunosubversion (protumor). We maintain that cancer immunoediting and tumor-promoting inflammation are not mutually exclusive processes, but rather are potentially overlapping immune algorithms (191).

This overlap was most clearly demonstrated in the MCA model in which sarcoma induction was shown to depend on immune cells and molecules that promote inflammation, including MyD88, IL-1β, IL-10, IL-23, and Tregs (34, 58, 117, 192, 193), but then led to the development of host-protective immune responses that resulted in tumor destruction (e.g., IFN-γ, IFN-α/β, T cells). For example, the functionally related heterodimeric cytokines IL-23 and IL-12 both contain the IL-12p40 subunit but activate distinct receptors that share the IL-12Rβ1 subunit and play different roles in response to transformed cells. Specifically, loss of IL-23 reduced the incidence of MCA-induced sarcomas, whereas IL-12-deficient mice developed more sarcomas when compared to wild-type mice (58).

Similarly, the DMBA/TPA model of skin carcinogenesis is known to have a major inflammatory component contributing to tumor development; however, γδ T cells, IL-12, and DNAM-1 participate in immunosurveillance and prevent skin carcinoma formation (21, 38, 57). Therefore, tumor-promoting inflammation and cancer immunosurveillance can coexist within the same tumor models at the same tissue site, although they may be temporally distinct. For example, both MyD88 and IL-1β have been shown to promote tumorigenesis in a number of primary carcinogen models (34, 192, 194, 195), but MyD88 and IL-1β are also critical in the development of antitumor immunity against established tumors through the recognition of dying tumor cells undergoing immunological death (196–198). Furthermore, the same component of the immune system may promote or prevent tumor formation depending on the biological context in which it acts. For example, mice genetically deficient for TNF-α develop more sarcomas than wild-type mice after exposure to MCA (34), indicating a host-protective role for this cytokine, whereas TNF-α-deficient mice develop fewer skin carcinomas than wild-type mice after exposure to DMBA/TPA (49), indicating a tumor-promoting role for TNF-α. One mechanism for TNF-α's ability to protect the host against tumor formation is the priming, proliferation, and recruitment of tumor-specific T cells that was observed in an oncogene-driven pancreatic cancer model (199).

Finally, inflammation participates in the cancer immunoediting process during the tumor escape phase, when inflammatory cells and regulatory immune cells are recruited and activated by cancer-derived products to dampen antitumor immunity and subvert immune cells to promote cancer progression. To develop more effective immunotherapies, immunologists must identify the cellular and molecular players that either eliminate or promote cancer development and what conditions influence that fate. For this reason, inhibitors of the proinflammatory transcription factors NF-κB and STAT3 may be therapeutically useful in switching the nature of the tumor microenvironment from one of tumor-promoting inflammation to that of tumor-eliminating immunity (200, 201).

LESSONS FROM CANCER IMMUNOEDITING

As our molecular understanding of cancer immunoediting increases, strategies can be developed to harness the power of immunity to protect against cancer development. Targets for therapeutic intervention can be found at each stage of the immunoediting process from elimination to equilibrium to escape. The identification of key immune molecules and cells important in the elimination of nascent

transformed cells may provide opportunities to boost specific aspects of immunity to induce tumor regression. Furthermore, development of therapeutic strategies that stabilize tumor masses by inducing an equilibrium state is a viable clinical endpoint that has not been fully implemented by oncologists but could greatly enhance patient survival. Other potential strategies targeting the equilibrium phase are those that attempt to stabilize tumor cell genetic instability, thereby halting progression from tumor equilibrium to tumor escape. The inhibition of tumor escape mechanisms may render tumor cells visible for immune-mediated destruction, and many pharmacological agents have been generated for this end.

Targets of tumor escape mechanisms currently in clinical trials or in the pipeline include antibody blockade of the immunosuppressive moieties CTLA-4, PD-L1, and PD-1. In the case of CTLA-4 blockade, a recent Phase 3 clinical trial reported that patients with metastatic melanoma survived longer after treatment with CTLA-4 blocking antibodies, making this drug one of the most successful cancer therapies that target the immune system (202). Furthermore, strategies to inhibit immunosuppressive cytokines such as VEGF, enzymes such as IDO, and antiapoptotic molecules such as Bcl-2 are also being pursued. Undoubtedly, chronic inflammation contributes to both cellular transformation and tumor progression, but less is known about what aspects specifically induce cancer formation. Inhibitors of proinflammatory transcription factors may reduce tumor development and switch the tumor microenvironment from tumor-promoting inflammation to tumor-eliminating immunity.

Ultimately, high-throughput screening of cancer genomes and proteomes is required to identify polymorphisms and mutations in immune pathways that limit human cancer development and progression. Insights gained from deciphering the molecular underpinnings of the cancer immunoediting process could lead to strategies for manipulating the cellular and molecular microenvironment of tumors in the hope of inducing immune-mediated eradication or stabilization of malignant disease.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize in advance to all the investigators whose research could not be appropriately cited due to space limitations. M.D. Vesely is supported by a predoctoral tumor immunology fellowship from the Cancer Research Institute. M.H. Kershaw is supported by a National Health and Medical Research Council of Australia (NHMRC) Senior Research Fellowship and project grants from the NHMRC and Cancer Council of Victoria. R.D. Schreiber is supported by NIH grants from the National Cancer Institute, the Cancer Research Institute, and the Ludwig Institute for Cancer Research. M.J. Smyth is supported by a NHMRC Australia Fellowship and Program Grant and a grant from the Association for International Cancer Research.

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8. First comprehensive description of concept of cancer immunoediting with a focus on the historical evolution of the concept.

14. Original demonstration that IFN-γ plays a critical function in cancer immunosurveillance.

17. Reveals the host-protecting and tumor-sculpting functions of immunity and introduces the concept of cancer immunoediting.

18. First demonstration that lymphocyte-mediated cytotoxicity protects the host from lymphoma development.

19. Demonstrates the importance of host NKT cells in the protection against carcinogenesis.

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Annual Review of Immunology
Volume 29, 2011

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